ANIMAL SCIENCE (ZOOTECNIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.18, n.4, e0028, 2023
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v18i4a0028 - Protocol 0028
Subm. 28/04/2022 • App. 30/12/2023 • Pub. 31/12/2023

DNA polymorphism of *Desmanthus* spp. accessions based on AFLP markers

Ildja Viviane de Queiroz[1], Mércia Virginia Ferreira dos Santos[1]*, James Pierre Muir[2], José Carlos Batista Dubeux Junior[3], Éderson Akio Kido[4], Márcio Vieira da Cunha[1], José Henrique Albuquerque Rangel[5], Alexandre Carneiro Leão de Mello[1]

[1] Federal Rural University of Pernambuco, Recife, PE, Brazil. E-mail: ildjaviviane1@gmail.com; mercia.vfsantos@ufrpe.br; marcio.cunha@ufrpe.br; alexandre.lmello@ufrpe.br
[2] Texas A&M Agrilife Research and Extension Center at Stephenville, Stephenville, TX, United States of America. E-mail: jim.muir@ag.tamu.edu
[3] University of Florida, North Florida Research & Education Center, Marianna, FL, United States of America. E-mail: dubeux@ufl.edu
[4] Federal University of Pernambuco, Recife, PE, Brazil. E-mail: kido.ufpe@gmail.com
[5] Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil. E-mail: jose.rangel@embrapa.br

ABSTRACT: Germplasm collections are key to assess genetic variability of forage germplasm. The objective of the present work was to study the amplified fragment length polymorphism (AFLP) presented by five *Desmanthus* spp. accessions. The study assessed the AFLP polymorphism of *Desmanthus pernambucanus* (L.) Thellung in Pernambuco, Brazil, and *D. pubescens* B.L. Turner AusTRC from Saint Croix, U.S. Virgin Islands. The 38 primers pairs tested resulted in 32 combinations and amplified 707 amplicons, of which 436 (62%) were polymorphic and 271 (38%) monomorphic, indicating a wide variability among the five *Desmanthus* accessions. Nei's genetic distance matrix pointed that 89F and AusTRC, distinct species collected from different locations (Bom Jardim and Saint Croix, USA), presented lesser genetic distance (33.5%) than 43F and 100C, belonging to *D. pernambucanus* but collected in Bom Jardim and Sertânia, respectively, with 57.1% distance. *D. pernambucanus* accessions 43F and 89F were the closest geographically located, however, presented expressive genetic distance (63.0%). The genetic variability of native germplasm could become important in increasing genetic adaptation and environmentally viable forage species.

Key words: genetic variability; germplasm; native legumes

Polimorfismo de DNA de acessos de *Desmanthus* spp., baseado em marcadores AFLP

RESUMO: Coleções de germoplasma são importantes para caracterização da variabilidade genética. O objetivo deste trabalho foi estudar o polimorfismo de comprimento de fragmentos amplificados (AFLP) apresentados por cinco acessos de *Desmanthus* spp. Um polimorfismo AFLP de *Desmanthus pernambucanus* (L.) Thellung foi realizado em Pernambuco, Brasil, e *D. pubescens* B.L. Turner AusTRC de Saint Croix, Ilhas Virgens Americanas. Foram testados 38 pares de primers, 32 combinações amplificadas e 707 amplicons, sendo 436 (62%) polimórficos e 271 (38%) monomórficos, indicando grande variabilidade entre os cinco acessos. A matriz de distância genética de Nei mostrou que 89F e AusTRC, espécies distintas coletadas em locais diferentes (Bom Jardim e Saint Croix, EUA), apresentaram menor distância genética (33,5%) do que 43F e 100C, pertencentes a *D. pernambucanus*, coletadas em Bom Jardim e Sertânia, respectivamente, com 57,1% de distância. Os acessos geograficamente mais próximos de *D. pernambucanus*, 43F e 89F, apresentaram uma distância genética significativa (63,0%). A variabilidade genética de germoplasma nativo pode tornar-se importante para aumentar a adaptação genética e espécies forrageiras ambientalmente viáveis.

Palavras-chave: variabilidade genética; germoplasma; leguminosas nativas

* Mércia Virginia Ferreira dos Santos - E-mail: mercia.vfsantos@ufrpe.br (Corresponding author)
Associate Editor: Mário de Andrade Lira Júnior

Introduction

The genus *Desmanthus*, belonging to the Fabaceae family, includes 23 species naturally distributed in tropical and subtropical regions of the Americas, with the highest diversity in Mexico (14 species) and southern Texas (8 species) (Luckow, 1993; Muir et al., 2014). The legume characteristic allows it to be considered an alternative feed source for ruminants, mainly in semi-arid areas (Gardiner & De Cat, 2014; Calado et al., 2016; Santos et al., 2019). The association of the bacteria with the nitrogen-fixing Rhizobiaceae family (Beyhaut et al., 2006; Freitas et al., 2011) allows intercropping of this legume with low nitrogen-fixing cultivars. Besides that, native plants are of great interest because are ecologically adapted (Silva et al., 2018; Tedesco et al., 2019; Almeida et al., 2019).

Most *Desmanthus* species are diploid with 2n = 28 chromosomes (Hughes et al., 2003). More recent studies by Santos et al. (2012) cited the species *Desmanthus pernambucanus* with 2n = 26 chromosomes. *Desmanthus* spp. presents a high degree of intra- and interspecific polymorphism (Luckow, 1993; Hughes et al., 2003), and considering the importance of genetic variability as a source of genes of interest, it is clear the importance of species conservation. Thus, the collection and maintenance of a representative set of genotypes of *Desmanthus* spp. in germplasm banks are essential to minimize the effects caused, for example, by the loss of genetic variability. Therefore, information about the plant development and genetic variation of species, especially native species, is fundamental for the domestication and incorporation of these species in the productive systems of a particular region. Furthermore, the development of efficient conservation strategies is necessary, and are closely related to knowledge of the magnitude and distribution of genetic variability in natural populations (Schultze-Kraft et al., 2020).

Consequently, to characterize the genetic variability of plants, especially from germplasm banks, molecular markers studies are performed. Some markers detect the genetic polymorphism directly in the deoxyribonucleic acid (DNA), without the influence of the environment. The molecular marker based on amplified fragment length polymorphism (AFLP) has been widely applied in studies of plant genetic diversity. The AFLP markers access variability at the DNA level after enzymatic DNA digestion followed by PCR (Polymerase Chain Reaction) amplification without the need for prior sequence information (Vos et al., 1995). The great advantage of the AFLP technique is the high number of generated markers, possibly a hundred, after combining primers derived from restriction sites of two enzymes (a frequent cutter and a rare cutter). Thus, ALFP is a viable alternative method to estimate the genetic variability of *Desmanthus* spp. accessions, since there are no specific primers derived from nucleotide sequences available in public databases.

Determining and understanding genetic variability in plant germplasm is the starting point for pre-breeding and breeding programs. This extensive characterization is necessary in order to fully explore the genetic variability in a valuable way to develop new forage cultivars. The molecular results provide information on the genetic variability of accessions and may indicate the need for interchange and introduction of new genotypes. According to Dubeux Jr et al. (2022), native legumes have high potential for different uses and still lack more detailed studies.

Thus, the main objective of the present work was to study the AFLP polymorphisms presented by five *Desmanthus* spp. accessions of the Germplasm Bank at the Federal Rural University of Pernambuco (UFRPE).

Materials and Methods

The molecular assay conducted in the laboratory of Molecular Genetics of the Federal University of Pernambuco - UFPE included five accessions of *Desmanthus* spp. Among these five accessions, four *Desmanthus pernambucanus* accessions (7G, 100C, 43F, and 89F), classified by the herbaria of the Instituto Agronômico de Pernambuco, Fomento Internacional do Brasil Ltda (FIB) nº 02/2012, from the Germplasm Bank of the Federal Rural University of Pernambuco-UFRPE, located in Serra Talhada-PE, collected in the municipalities of Santa Cruz do Capibaribe, Sertânia and Bom Jardim (Queiroz, 2012). The fifth accession was from Embrapa Coastal Trails - Sergipe (originally from the Australian germplasm bank - CSIRO, Austr), identified as *Desmanthus pubescens* B.L. Turner (Table 1).

The number of accessions analyzed in the study considered the low survival of plants in the *in vivo* germplasm bank, installed in the municipality of Serra Talhada since 2010. The annual precipitation (mm) presented in that area covered 682.0 (2010), 644.1 (2011), 163.3 (2012), 598.3 (2013), 708.6 (2014), and 395.4 mm (2015) (APAC, 2021).

Table 1. Relationship of *Desmanthus* spp. evaluated in the AFLP assay, with their respective geographical origins and coordinates.

Identification	Origin	Latitude	Longitude	Soil type
100C*	Sertânia – Brasil (BR)	08º04'S	37º18'W	LUV
43F*	Bom Jardim – BR	07º47'S	35º32'W	AVE
89F*	Bom Jardim – BR	07º50'S	35º41'W	VER
7G*	Santa Cruz do Capibaribe – BR	07º50'S	36º22'W	NR
AusTRC**	U.S. Virgin Islands – CSIRO	18º08'N	61º68'W	-

* From the Germplasm Bank of the Federal Rural University of Pernambuco - UFRPE; **AusPGRIS – Australian Plant Genetic Resource Information Service (http://www2.dpi.qld.gov.au/extra/asp/AusPGRIS/Scripts/Display_Accession.asp?theAccession=AusTRCF92803). LUV = luvisol; AVE = dark red argisol; VER = vertisol; NR = regolitic neossol.

Plants were propagated by seeds submitted to breakage of dormancy by cutting (with scissors cut in the region opposite the seed thread) (Queiroz, 2012) and sowed in pots (3 kg). After 30 days of plant development (5 plants/access), young leaves were collected, immediately frozen in liquid nitrogen, and stored (-80 °C) until DNA extraction.

The samples (200 mg) were ground in liquid nitrogen using a porcelain mortar and pestle, with 80 µL of 2% CTAB (NaCl 5M, Tris HCl 1 M pH 8.0; EDTA 0.5 M). The buffer was the cetyl trimethyl ammonium bromide and 20 µL of β-mercaptoethanol, proposed by Doyle (1991) and modified by Silva et al. (2008). Organic extraction was performed with chloroform:isoamyl alcohol 24:1 to remove the contaminants present in the DNA solution, and the DNA precipitated with ammonium acetate at 7.5 M and 95% ethyl alcohol (1:6). Then, the excess of salt was washed off the DNA with 75% ethanol. The precipitated DNA was diluted in 100 µL TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), quantified on 0.8% agarose gel stained with 0.02% ethidium bromide solution (10 mg/ml) and visualized in ultraviolet light transilluminator.

The AFLP reactions were developed using the AFLP® Analysis System I kit (Life Technologies®), according to the manufacturer's instructions. In summary, following digestion with the *Eco*RI and *Mse*I enzymes and ligation of specific adapters, the DNA was amplified using primers with three selective bases (*Eco*RI-NNN and *Mse*I-NNN), and for the primers, each N corresponds to a nucleotide: A, C, T or G. PCR reactions were performed on PTC-100 thermocycler (MJ Research), following the AFLP kit instructions. The amplicons were visualized after 6% polyacrylamide gel electrophoresis in a denaturing condition (7M urea), using a vertical gel apparatus, with 1x TBE buffer. Gel staining with silver nitrate followed the protocol described by Bassam et al. (1991).

The results were converted into a binary matrix, based on the presence (1) or absence (0) of the amplicon. The percentage of polymorphic loci and the optimal number of fragments were calculated with the aid of the Genes program (Cruz, 2006). The number of alleles observed (Na), number of effective alleles (Ne), and heterozigosidade (H) were performed using the PopGene software (v.1.31), as well as the UPGMA ("unweighted pair group method using arithmetic averages") dendrogram based on Nei's genetic distances (Nei, 1978) involving the different accessions of *Desmanthus* spp.

Results

Of the 38 primer combinations tested to characterize five accessions of the genus *Desmanthus*, 32 combinations amplified 707 amplicons (Table 2), of which 436 (62%) were polymorphic (presence or absence of the amplicon in at least one of the accessions) and 271 (38%) common to the five accessions (Figure 1), which evidenced the variability among them, and the polymorphism detection power presented by the AFLP technique in *Desmanthus* spp.

Table 2. Amplified and polymorphic amplicons generated using AFLP primer combinations with DNAs of *Desmanthus* spp.

Initiators *	Total amplified bands	Total polymorphic fragments	Polymorphism (%)
E-ACG + M-CAA	34	25	74
E-ACG + M-CTC	28	16	57
E-ACG + M-CTG	38	32	84
E-ACG + M-CTT	24	09	38
E-ACG + M-CTA	20	14	70
E-ACG + M-CAG	13	10	77
E-ACG + M-CAT	17	16	94
E-AGC + M-CTC	15	09	60
E-AGC + M-CTG	03	02	67
E-AGC + M-CTT	35	30	86
E-AGC + M-CTA	05	02	40
E-AGC + M-CAT	11	05	45
E-AGC + M-CAC	07	01	14
E-ACC + M-CAA	15	11	73
E-ACC + M-CTC	13	06	46
E-ACC + M-CTG	20	09	45
E-ACC + M-CTA	23	13	56
E-ACC + M-CAG	27	07	26
E-ACC + M-CAC	33	18	55
E-AAC + M-CAA	07	00	00
E-AAC + M-CTC	25	16	64
E-AAC + M-CTG	10	06	60
E-AAC + M-CTT	52	33	63
E-AAC + M-CTA	20	08	40
E-AAC + M-CAG	22	13	59
E-AAC + M-CAT	24	24	100
E-AAC + M-CAC	21	21	100
E-ACT + M-CAA	40	13	33
E-ACT + M-CTG	26	14	35
E-ACT + M-CTT	32	17	53
E-ACT + M-CAG	25	23	92
E-ACT + M-CAC	22	13	59
TOTAL	707	436	62
Mean	22	14	

* Selective bases starting with A refer to primers for EcoRI and those with C are from MseI.

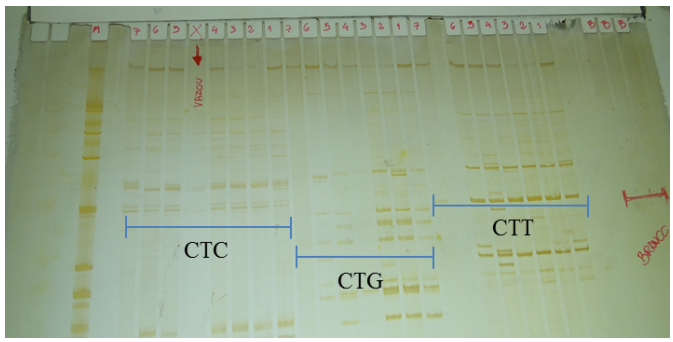

Figure 1. Amplicons (polymorphic and monomorphic) amplified with DNAs of five accessions (*Desmanthus* spp.) visualized after silver staining of the 6% polyacrylamide gel. * 1 = 7G; 2 = 100C; 3 = 89F; 4 = NSDD; 5 = AusTRC; 6 = 43F; 7 = 21F. ** NSDD and 21F were not used per problem in execution. *** CTC = M-CTC; CTG = M-CTG; CTT = M-CTT.

Each pair of primers produced between one and 33 distinguishable polymorphic amplicons with an average of 14 polymorphic fragments per primers-pair combination, while the total amplicons ranged from three to 52 with a mean of 22 per primers-pair (Table 2). The E-AAC/M-CTT primers-pair was the one with the highest number of amplified and polymorphic amplicons, whereas the E-AAC/M-CAA combination did not present polymorphic fragments. Of the 38 primers-pair combinations tested, six did not amplify the DNAs: E-AGC/M-CAG; E-ACC/M-CTT; E-ACC/M-CAT; E-ACT/M-CTC; E-ACT/M-CTA; E-ACT/M-CAT.

With the genetic distance matrix of Nei (1978), which measures genetic diversity among different species or individuals of the same species, a dendrogram was constructed using the UPGMA method (Figure 2). The dendrogram calculated from Nei's unbiased genetic distances between populations by the UPGMA method revealed two distinct clusters.

The unbiased genetic Cluster 1 comprises accessions 7G e 100C, originating from Santa Cruz do Capibaribe and Sertânia, respectively. Cluster 2 includes accessions 89F and 43F, both from Bom Jardim, and the AusTRC accession from U.S. Virgin Islands (Table 1). The lowest genetic distance occurred between the 89F and AusTRC accessions (33.5%), from Bom Jardim and Saint Croix (U.S. Virgin Islands), respectively (Table 3). The highest distance was observed between accesses 43F and 100C (57.1%), from Bom Jardim and Sertânia, respectively.

The genetic identity matrix showed the range of similarity, varying from 56.5% to 71.5% between the accessions. These values indicated that the 89F and AusTRC accessions were the most genetically similar (Ig: 0.7153), which does not mean that they belong to the same species.

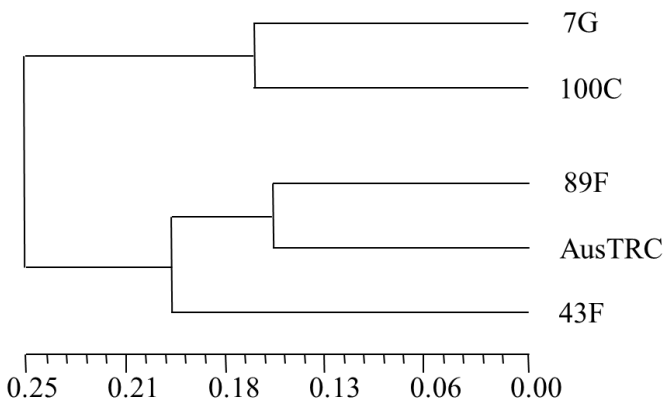

Figure 2. Dendrogram considering accessions of *Desmanthus* spp.

Genetic variation, employing the AFLP markers for allele frequency, effective allele numbers, Shannon index, and genetic diversity of Nei (1978) (Table 4), allowed to observe that the average number of alleles per locus was 1.70, and the effective number of alleles, which contributed to genetic diversity, was 1.51.

According to Nei (1978), the average number of alleles per locus (A) is greatly influenced by the size of the genetic sample (loco number) in the collection. The heterozygosity (H), also called the Nei genetic index, was used to evaluate the polymorphic content of each locus and obtained a value of 0.29. The Shannon Index was 0.42, considered moderate to low (Table 4).

Table 4. Estimates of the number of alleles, Shannon Index and genetic diversity based on 707 AFLP markers in *Desmanthus* spp.

Parameter	Core collection	
	Average	Deviation
Number of alleles observed (Na)	1.70	0.46
Number of effective alleles (Ne)	1.51	0.38
Heterozygosity (H)	0.29	0.20
Shannon Index (I)	0.42	0.28

Discussion

Based on the level of polymorphism reached (Figure 1), it will be possible to discriminate genotypes at the DNA level, planning crosses in plant genetic improvement, considering the agronomic performance of the parents in the most advanced stages of selection and breeding strategies. Pengelly & Liu (2001), evaluating 284 accessions of *Desmanthus* spp., equivalent to 11 species, from analyzes with Random Amplified Polymorphic DNA (RAPD), observed that there was a higher polymorphism in *D. virgatus*, *D. leptophyllus*, and *D. pernambucanus*, concerning *D. pubescens*. Silva et al. (2023) too observed higher polymorphism, selected primers amplified 38 fragments, with 71.05% polymorphism. The highest polymorphism percentage was shown by ISSR13 (100%), and the lowest by ISSR12 which presented only monomorphic fragments (0% polymorphism).

The highest number of accessions worked by those authors allowed data comparisons by species, unlike the present study with only five accessions, highlighting the issue of intensifying the works incorporating more accessions and species, making it possible to screen all accessions from a germplasm active bank. It is worth highlighting the difficulty of maintaining an *in vivo* germplasm bank in a semi-arid

Table 3. Genetic identity (Ig) and genetic distance (Dg) in accessions of *Desmanthus* spp.

Accessions	7G	100C	89F	AusTRC	43F
7G	****	0.7068	0.6133	0.5836	0.5807
100C	0.3470	****	0.6997	0.6190	0.5652
89F	0.4889	0.3571	****	0.7153	0.6303
AusTRCF92803	0.5386	0.4797	0.3351	****	0.6997
43F	0.5435	0.5707	0.4615	0.3571	****

* Genetic identity: values above the diagonal; Genetic distance: values below the diagonal.

environment, which limits the availability of accessions for evaluations. Despite the few accessions evaluated in the present study, the genetic variability was estimated based on AFLP markers, whose technique is more robust and reproductive than the RAPD technique applied by the authors previously mentioned (Gardiner & Burt, 1995).

Despite the proximity of the place where the accessions were collected (municipalities), there is high variability among them (Figure 1), even being plants with autogamous reproduction (Luckow, 1993). The genetic variability observed among the accessions allows adaptation to the environmental changes (Daufresne & Renault, 2006), which highlights the importance of the preservation of *Desmanthus* accessions through the germplasm banks, both for biological conservation and plant genetic improvement.

Similar results were found by Costa et al. (2017), who observed high variability in 22 accessions of the UFRPE germplasm active bank, but only 15 ISSR markers were analyzed. The ISSR markers are also dominant markers, like those AFLP and RAPD markers (Turchetto-Zolet et al., 2017). Besides, the authors mentioned eight clusters grouped by the ISSR markers, and they also reported high variability in accessions collected in nearby locations, and some molecular proximity of those accessions collected from more distant places. It is noteworthy that after the monograph of *Desmanthus* reported by Luckow (1993), it became evident that most of the germplasm previously identified as *D. virgatus* was, in fact, *D. pernambucanus* (L.) Thell (Cook & Schultze-Kraft, 2015).

Silva et al. (2023) working with *Desmanthus* observed values close to those found in the present study (Table 4), where the Shannon Index also corresponded to 0.42. A moderate (0.361) Shannon Index value was observed in studies of diversity of accessions of *Desmanthus pernambucanus*, carried out by Costa et al. (2017).

The observed heterozygosity is an index of genetic diversity greatly influenced by the reproductive system of the species. A natural population of allogeneic species exhibits higher heterozygosity than an autogamous species population.

According to Dubeux Jr et al. (2022), the preservation of native legume species is of fundamental importance not only for maintaining the biodiversity of the Caatinga, but also for preserving the sustainability of this important biome. Santos et al. (2022) mention that studies that measure the qualitative and quantitative variations of native Caatinga forages are essential for the management of food supplementation, with a view to the sustainability of animal production in Caatinga areas, in addition to gains in the preservation of this ecosystem.

Conclusion

Accessions (7G, 100C, 89F, 43F, and AusTRC) present high DNA polymorphisms, evidencing the efficiency of the AFLP technique in evaluating the genetic variability of *Desmanthus* spp.

Genetic distance is not limited by the geographic distance since accessions geographically close and belonging to the same species (89F and 43F) did not show superior genetic similarities, although accessions geographically distant, and eventually of different species (89F and AusTRC) may share expressive genetic similarities. The 43F and 100C accessions are relatively distant genetically from each other, being important sources of diversity in the studied germplasm.

The existence of some unique accessions reinforces the importance of preserving native plant populations, looking for valuable features and resources for plant breeding programs.

Acknowledgements

The team of the Laboratory of Molecular Genetics of the Federal University of Pernambuco - UFPE, for the support in the analysis of DNA extraction and evaluation. To CNPq and FACEPE for the financial support of the research and fellowships. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Compliance with Ethical Standards

Authors contributions: Conceptualization: MVFS, MVC, JCBDJ, ACLM; Data curation: MVFS; Formal analysis: IVQ, MVC, JPM; Funding acquisition: MVFS; Investigation: IVQ, MVFS, MVC, EAK, JPM; Methodology: MVFS, MVC, JCBDJ, ACLM, JHAR; Project administration: MVFS, MVC, JCBDJ, ACLM; Resources: MVFS, EAK; Supervision: MVFS, MVC; Validation: IVQ, MVFS, MVC, JHAR; Visualization: MVFS, IVQ; Writing – original draft: IVQ, MVFS, MVC; Writing – review & editing: MVFS, MVC, EAK, JCBDJ, JPM, ACLM.

Conflicts of interest: The authors declare no conflict of interest.

Funding source: The Coordination for the Improvement of Higher-Level Personnel – Brazil (CAPES) - Financing Code 001; the National Council of Scientific and Technological Development (CNPq) – Grants 312610/2021-1; the Bank of Northeast Brazil (BNB), Science; the Technology Support Foundation of Pernambuco State (FACEPE) and the Federal Rural University of Pernambuco (UFRPE).

Literature Cited

Almeida, G. Q.; Chaves, L. J.; Vieira, M. C.; Ganga, R. M. D. Agronomic evaluation of a *Hancornia speciosa* Gomes germplasm collection from the Brazilian Cerrado. Crop Breeding and Applied Biotechnology, v. 19, n. 1, p.8-14, 2019. https://doi.org/10.1590/1984-70332019v19n1a02.

Agência Pernambucana de Águas e Climas - APAC. Meteorologia. Monitoramento pluviométrico. http://old.apac.pe.gov.br/meteorologia/monitoramento-pluvio.php. 12 Apr. 2022.

Bassam, B. J.; Caetano-Anollés, G. E.; Gresshoff, P. M. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry, v. 196, n. 1, p.80-83, 1991. https://doi.org/10.1016/0003-2697(91)9,0120-I.

Beyhaut, E.; Dehaan, L.R.; Byun, J.L.; Sheaffer, C.C.; Graham, P.H. Response to inoculation in *Illinois bundleflower*. Canadian Journal of Plant Science, v. 86, n. 4, p.919–926, 2006. https://doi.org/10.4141/P05-097.

Calado, T.B.; Cunha, M.V.; Teixeira, V.I.; Santos, M.V.F.; Cavalcanti, H.S.; Lira, C.C. Morphology and productivity of "Jureminha" genotypes (*Desmanthus* spp.) under different cutting intensities. Revista Caatinga, v.29, n. 3, p.742-752, 2016. https://doi.org/10.1590/1983-21252016v29n326rc.

Cook, B. G.; Schultze-Kraft, R. Botanical name changes – nuisance or a quest for precision? Tropical Grasslands-Forrajes Tropicales, v. 3, n. 1, p.34–40, 2015. https://doi.org/10.17138/tgft(3)34-40.

Costa, J. C.; Fracetto, G. G. M.; Fracetto, F. J. C.; Santos, M. V. F.; Lira Jr, M. A. Genetic diversity of Desmanthus sp accessions using ISSR markers and morphological traits. Genetics and Molecular Research, v. 16, n. 2, gmr16029667, 2017. https://doi.org/10.4238/gmr16029667.

Cruz, C. D. Programa genes: biometria. Viçosa: Editora UFV, 2006. 382p.

Daufresne, M.; Renault, O. Population fluctuations, regulation and limitation in stream-living brown trout. Oikos, v. 113, n. 3, p.459-468, 2006. https://doi.org/10.1111/j.2006.0030-1299.14295.x.

Doyle, J. DNA Protocols for Plants. In: Hewitt, G.M.; Johnston, A.W.B.; Young, J.P.W. (Eds) Molecular Techniques in Taxonomy. Heidelberg: Springer, 1991. p 283–293. (NATO ASI Series, 57). https://doi.org/10.1007/978-3-642-83962-7_18.

Dubeux Jr, J. C. B.; Santos, M. V. F.; Macedo, I. M.; Muir, J. P.; Lira Jr, M. L Leguminosas Nativas. In: Santos, M. V. F.; Neiva, J. N. M. (Eds.). Culturas forrageiras no Brasil: uso e perspectivas. Visconde do Rio Branco: Suprema Gráfica, 2022. p.231-262. https://www.ppgz.ufrpe.br/media/2/download. 07 Dec. 2022.

Freitas, A. D. S.; Silva, T. O.; Menezes, R. S. C.; Sampaio, E. V. S. B.; Araújo, E. R.; Fraga, V. S. Nodulação e fixação de nitrogênio por forrageiras da caatinga cultivadas em solos do semiárido paraibano. Revista Brasileira de Zootecnia, v. 40, n. 9, p.1856-1861, 2011. https://doi.org/10.1590/S1516-35982011000900003.

Gardiner, C.; De Cat, S. New legumes for enhanced sheep/cattle production in dry tropical environments. In: The Australian Veterinary Association Annual Conference, 2014, Perth. Proceedings... Perth: Australian Veterinary Association, 2014. p.F1.4b.1-F1.4b.1. https://www.researchgate.net/publication/262640638. 09 Apr. 2022.

Gardiner, C. P.; Burt, R. L. Performance characteristics of *Desmanthus virgatus* in contrasting tropical environments. Tropical Grasslands, v. 29, n. 3, p.183-187, 1995. https://www.researchgate.net/publication/260885116. 21 Apr. 2022.

Hughes, C. E.; Bailey, C. D.; Krosnick, S.; Lucrow, M. A. Relationships among genera of the informal *Dischroschys* and Leucena groups (*Mimosoideae*) inferred from nuclear ribosomal ITS sequences. In: Klitgaard, B. B.; Bruneau, A. (Eds.). Advances in Legume Systematics. Kew: Royal Botanic Gardens, 2003. p.221-238. https://www.researchgate.net/publication/267255771. 09 Apr. 2022.

Luckow, M. Monograph of *Desmanthus* (Leguminosae-Mimosoideae). Systematic Botany Monographs, v. 38, p.1-166, 1993. https://doi.org/10.2307/25027822.

Muir, J. P.; Dubeux Jr, J. C. B.; Santos, M. V. F.; Maposse, I. C.; Pitman, W. D.; Butler, T. J. Challenges to domesticating native forage legumes. Tropical Grasslands – Forrajes Tropicales, v. 2, p.94-96, 2014. https://doi.org/10.17138/tgft(2)94-96.

Nei, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, v. 89, n. 3, p.583-590, 1978. https://doi.org/10.1093/genetics/89.3.583.

Pengelly, B. C.; Liu, C. J. Genetic relationships and variation in the tropical mimosoid legume *Desmanthus* assessed by random amplified polymorphic DNA. Genetic Resources and Crop Evolution, v. 48, p.91-99, 2001. https://doi.org/10.1023/A:1011234913710.

Queiroz, I. V. Ocorrência e germinação de sementes de *Desmanthus* sp. coletadas na semiárido pernambucano. Recife: Universidade Federal Rural de Pernambuco, 2012. 80p. Master's Thesis. https://www.ppgz.ufrpe.br/sites/default/files/testes-dissertacoes/ildja_viviane_de_queiroz.pdf. 21 Apr. 2022.

Santos, E. C. X. R.; Carvalho, R.; Almeida, E. M.; Felix, L. P. Chromosome number variation and evolution in Neotropical *Leguminoseae* (*Mimosoideae*) from northeastern Brazil. Genetics and Molecular Research, v. 11, n. 3, p.2451-2475, 2012. https://doi.org/10.4238/2012.June.27.1.

Santos, M. V. F.; Cunha, M. V.; Dubeux Jr, J. C. B.; Ferreira, R. L. C.; Lira Jr, M. A.; Oliveira, O. F. Native shrub-tree legumes of tropical America with potential for domestication. Legume Perspectives - The Journal of the International Legume Society, n.17, p. 33–35. 2019. https://www.legumesociety.org/wp-content/uploads/2019/12/legum_perspect_17.pdf. 21 Apr. 2022.

Santos, M. V. F.; Souza, R. T. A.; Cunha, M. V.; Magalhaes, A. L. R. Potencial forrageiro de plantas da Caatinga. In: Santos, M. V. F.; Neiva, J. N. M. (Eds.). Culturas forrageiras no Brasil: uso e perspectivas. Visconde do Rio Branco: Suprema Gráfica, 2022. p.285-312. https://www.ppgz.ufrpe.br/media/2/download. 07 Dec. 2022.

Schultze-Kraft, R.; Cook, B. G.; Ciprián, A. Clearing confusion in *Stylosanthes* taxonomy. 2. *S. macrocephala* M.B. Ferreira & Sousa Costa cv. *S. capitata* Vogel and *S. bracteosa* Vogel. Tropical Grasslands-Forrajes Tropicales, v. 8, n. 3, p.250-262, 2020. https://doi.org/10.17138/tgft(8)250-262.

Silva, A. V. C.; Nascimento, I. M. R.; Rangel, J. H. A.; Muniz, E. N.; Pereira, C. S. A. Exchange of germoplasm and genetic diversity to enrichment the *Desmanthus* genebank. Revista Caatinga, v. 36, n. 1, p. 33-40, 2023. https://doi.org/10.1590/1983-21252023v36n104rc.

Silva, A.V.C.; Rangel, J.H.A.; Muniz, E.N.; Ragel, S.; Santos, A.R.F.; Aragão, W. M. Variabilidade genética entre acessos de jureminha (*Desmanthus virgatus* (L.) Wild). In: Congresso Nordestino de Produção Animal, 5., 2008, Aracaju. Anais... Aracaju: Sociedade Nordestina de Produção Animal; Embrapa Tabuleiros Costeiros, 2008.

Silva, D. C.; Blank, A. F.; Nizio, D. A. C.; Sampaio, T. S.; Nogueira, P. C. L.; Arrigoni-Blank, M. F. Chemical diversity of essential oils from native populations of *Eplingiella fruticosa*. Crop Breeding and Applied Biotechnology, v. 18, n. 2, p.205-214, 2018. https://doi.org/10.1590/1984-70332018v18n2n29.

Tedesco, M.; Emer, A. A.; Winhelmann, M. C.; Avrella, E. D.; Krycki, K. C.; Simioni, C.; Schafer, G. Cytogenetic characterization of *Angelonia integérrima* Sprengel, a native species with ornamental potential. Crop Breeding and Applied Biotechnology, v. 19, n. 1, p.118-125, 2019. https://doi.org/10.1590/1984-70332019v19n1a16.

Turchetto-Zolet, A. C.; Turchetto, C.; Zanella, C. M.; Passaia, G. 2017. Marcadores moleculares na era genômica: metodologias e aplicações. Ribeirão Preto: Sociedade Brasileira de Genética, 2017. 181p. https://www.lume.ufrgs.br/bitstream/handle/10183/206114/001056131.pdf?sequence=1. 21 Apr. 2022.

Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Van de Lee, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research v. 23, n. 21, p.4407-4414, 1995. https://doi.org/10.1093/nar/23.21.4407.